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A CLINICAL METHOD FOR THE QUANTITATIVE ESTIMATION OF URIC ACID IN URINE.

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VARIOUS methods for the estimation of uric acid in urine have been devised which are, however, only useful for laboratory workers, and require the use of several reagents and considerable technical skill. From amongst all the methods the simplest and fairly accurate one is the Gowland-Hopkins method; however, even this requires about 50 to 100 c.cm. of urine and various chemicals, and the result cannot be obtained within three to four hours at the earliest.

The following method, which I have found to work satisfactorily for over three years, will, I hope, recommend itself to others on account of its simplicity and fair amount of accuracy. For this method 5 to 10 c.cm. of urine is quite sufficient, and the only reagent required is a couple of tiny drops of pure hydrochloric acid; the drops must be so small that 50 such equal 1 c.cm. of the acid. This can be easily managed by using a capillary tube, drawn out in a blowpipe like the ordinary glass bristle used for bacteriological purposes.

THE METHOD.

Five c.cm. of the urine are placed in a percentage centrifugal tube and two drops of pure HCl mixed with it. This tube is now immersed in a freezing mixture of salt and ice for about fifteen to twenty minutes. This freezes the urine, and the urates and uric acid are deposited in a dense opaque mass along with other salts.

Even at this stage one can see whether the uric acid is scanty or not, as the frozen urine appears translucent when the urates are scanty, while it is milky, curdy, opaque even when the amount of uric acid is normal.

Now this tube is removed from the freezing mixture and rapidly centrifugalized till the whole of the mass is liquefied.

It must then be allowed to rest till it approaches the normal temperature of the room, then the percentage of the deposit is to be read off. This percentage is less than 0.5 per cent. when the amount of uric acid is less than 0.04 per cent. as estimated by Fokker's or Gowland-Hopkins's method.

In case there is much pus or albumen or any other deposit is present, it must be removed by boiling 10 c.cm. of the urine acidulated with a tiny drop of glacial acetic acid, and 5 c.cm. of the filtered urine is to be treated as above. The temperature of the freezing mixture is about -12° C. in a room at 25° C.

Some samples of urine contain a deposit of urates and uric acid; in such cases, especially when crystals of uric acid are in abundance, it is necessary to dissolve these by the help of caustic soda and heat. About 10 c.cm. of the urine (after mixing it thoroughly) is treated with strong caustic soda solution till red litmus paper just shows a faint blue tinge, then this is to be boiled. The phosphates are precipitated by the procedure, but it is of no importance, and it is not necessary to remove them by filtration, as the subsequent addition of HCl dissolves them. Take 5 c.cm. of this boiled urine and treat as above.

Some people pass urine which deposits uric acid crystals very rapidly, although there is no increase of uric acid. (See cases No. 5, 5a, 9 of Table B.)

Again, in cases where there is actual increase of uric acid, the method of freezing it as described above does not give a proportionate amount of deposit, unless the urine is alkalinized and boiled as described above. (See cases No. 11, 12, 12a, 18, 18a of Table C.)

Even after centrifugalizing the urine it is advisable to leave the tube in the freezing mixture for about eight hours, as much of the uric acid will crystallize out by that time, and thus one can distinguish between the deposit due to the uric acid and the amorphous mass of coagulated albumin or pus cells which may have passed through the filter paper by accident. (See No. 11, Table B.)

With this limitation to the usefulness of the method, the centrifugal method is fairly accurate, and the result is obtained within half an hour.

Although oxalates are soluble in HCl, the trace added is not enough to dissolve the crystals when they are in abundance, and a few of these are deposited in the tube, along with the uric acid, but they are so few that they do not appreciably interfere with the volume of the deposit of urates and uric acid.

I have noticed in most of the cases, when the oxalates are increased in the urine, that the supernatant fluid after centrifugalizing the tube remains slightly turbid, but it clears up as the temperature of the tube comes up to about 25° C. This happens even though there is neither pus nor albumin in the urine. In fact, in such cases one can with confidence expect the oxalates to be increased, and subsequent examination with the microscope, or with the chemical method, has confirmed the surmise. So this method helps in forming an opinion about the quantity of oxalates in the urine also.

Tests of Accuracy.

To test the accuracy of this method I have compared the results obtained by it with actual uric-acid estimation by Fokker's method in 54 cases.

Urine, when cooled to 15° C. for about six hours, gives a slightly reddish, copious, curdy deposit of amorphous urates in case there is an increase of uric acid. Phosphates and oxalates, when in excess, are also deposited, but the uratic

TABLE A.—Uric Acid Scanty.

No.	Uric Acid (Fokker).	Uric Acid Freezing + HCl.	Specific Gravity.	Colour.	Reaction	Urea.	Chlorides.	Phosphates.	Oxalates.	Indican.	Albumen.	Pus.	Sugar.	Deposit on Cooling the Urine.	Remarks.
1	0.004	No deposit	1005	Pale yellow	Acid	Per cent.	—	Per cent.	—	—	Nil	Nil	Nil	Scanty	—
2	0.002	"	1034	Yellow	"	1.74	One-sixteenth	10	Not increased	Trace	"	"	7.5 per cent.	"	Crystals of uric acid present.
3	0.016	Slight deposit	1013	"	"	—	—	20	"	—	"	"	Nil	"	—
4	0.002	"	1009	"	"	1.07	One-twelfth	10	"	Trace	"	"	Nil	"	—
5	0.002	No deposit	1016	Pale yellow	1.5 ^N NaOH	—	—	—	—	—	"	"	"	"	—
6	0.014	Slight deposit	1020	"	NaOH Acid	—	—	—	—	—	3 per cent.	Copious	"	—	—
7	0.005	"	1020	Yellow	"	—	—	30	Copious	Trace	0.35 per cent.	Trace	"	Moderately increased	Deposit due to pus and oxalates.

TABLE B.—Uric Acid Moderate.

No.	Uric Acid (Fokker).	Uric Acid Freezing + HCl.	Specific Gravity.	Colour.	Reaction	Urea.	Chlorides.	Phosphates.	Oxalates.	Indican.	Albumen.	Pus.	Sugar.	Deposit on Cooling the Urine.	Remarks.
1	0.050	Slight deposit	1035	Yellow	Acid	Per cent.	—	Per cent.	—	—	Nil	Nil	6.25 per cent.	Scanty	—
2	0.037	"	1020	"	"	—	—	—	—	—	"	"	Nil	"	—
3	0.040	0.5 per cent.	1011	Deep yellow	"	1.07	One-fifth	20	Not increased	Trace	1 per cent.	"	"	"	—
4	0.050	0.3 per cent.	1015	Pale yellow	1.1 ^N NaOH	—	—	—	—	—	"	"	"	"	—
5	0.030	Slight deposit	1019	Yellow	2.6 ^N NaOH	—	—	—	—	—	"	"	"	Moderate	Crystals of uric acid present. Not treated with NaOH before adding HCl.
5a	0.047	0.3 per cent.	1019	"	NaOH	—	—	—	—	—	"	"	"	—	Crystals of uric acid present. Treated with NaOH before adding HCl.
6	0.037	Slight deposit	1015	"	Acid	—	—	—	Not increased	—	—	0.25 per cent.	"	Scanty	—
7	0.030	0.2 per cent.	—	—	Slightly acid	—	—	—	—	—	Nil	"	"	—	—
8	0.040	Slight deposit	1022	Yellow	Acid	2.95	One-tenth	24	Moderately increased	Moderate	"	"	"	Scanty	—
9	0.045	"	1020	"	"	—	—	—	—	—	"	"	"	"	Crystals of uric acid present. Not treated with NaOH before adding HCl.
10	0.043	No deposit at 15° C.	1035	"	2.3 ^N NaOH	—	—	—	—	—	"	"	4.63 per cent.	"	HCl plus freezing method not applied.
11	0.053	*1 per cent.	1023	Deep yellow	3.3 ^N NaOH	2.52	One tenth	30	Not increased	Trace	2 per cent.	"	Nil	"	Urobilin copious. *Casts in large number, and much of the deposit was due to the albumen, etc.
12	0.050	0.5 per cent.	1019	Yellow	"	—	One-sixteenth	10	Slightly increased	—	Nil	"	"	—	Carbonates present.
13	0.046	"	1018	"	slightly acid	1.34	One-tenth	6	Increased	Trace	"	"	"	Moderately increased	Carbonates excessive. The deposit on simply cooling the urine showed amorphous phosphates.
14	0.053	"	1045	"	Acid	—	—	—	—	Trace	"	"	6.25 per cent.	Scanty	Urobilin increased. Free.
15	0.040	"	1012	Pale yellow	"	—	—	6	Slightly increased	Moderately increased	"	"	Nil	"	—
16	0.035	"	1036	Yellow	"	—	—	30	—	—	"	"	6.25 per cent	—	—

TABLE C.—Uric Acid Copious.

No.	Urie Acid (Fokker).	Urie Acid Freezing + HCl.	Specific Gravity.	Colour.	Reaction	Urea.	Chlorides.	Phos- phates.	Oxalates.	Indican.	Albumen.	Pus.	Sugar.	Deposit on Cooling the Urine.	Remarks.
1	Per cent. 0.085	1.50 per cent.	1025	Deep yellow	Acid	Per cent. —	—	Per cent. —	—	—	Nil	Nil	Trace	Scanty	—
2	0.084	1.50 per cent.	1020	"	"	—	—	—	—	—	"	"	"	"	Urobilin free (trace).
3	0.090	1.00 per cent.	1030	Yellow	3.6 N NaHO Acid	3.97	One-tenth	20	Not increased	Moderately increased	"	"	Nil	Copious	—
4	0.065	0.75 per cent.	1030	"	Acid	—	—	—	—	—	"	"	1.6 per cent.	Scanty	—
5	0.100	1.00 per cent.	1027	"	"	—	—	30	Increased	—	"	"	Nil	"	—
6	0.080	1.00 per cent.	1027	"	"	—	—	—	—	Trace	"	"	"	Copious	—
7	0.080	1.00 per cent.	1036	Deep yellow	"	—	—	—	—	—	"	"	"	"	—
8	0.080	1.00 per cent.	1033	"	"	—	—	—	—	—	"	"	"	"	—
9	0.085	0.50 per cent.	1035	Yellow	"	—	—	—	—	—	"	"	per 3 cent.	Scanty	Probably not frozen.
10	0.082	1.00 per cent.	1037	Deep yellow	"	—	—	—	—	—	"	"	Trace	"	—
11	0.070	slight deposit	1023	Yellow	5.8 N NaHO	3.35	One-tenth	20	Slight increase	Trace	"	"	Nil	"	Crystals of uric acid present. Not treated with NaHO before adding HCl.
12	0.060	0.50 per cent.	1030	"	5.0 N NaHO	—	—	40	Increased	—	"	"	"	"	Crystals of uric acid present. Not treated with NaHO before adding HCl.
12a	0.060	1.00 per cent.	1030	"	"	—	—	40	"	—	"	"	"	"	Crystals of uric acid present. Treated with NaHO, etc., before adding HCl.
13	0.060	1.00 per cent.	1025	Deep yellow	3.8 N NaHO	—	—	40	Copious	—	0.35 per cent.	Trace	"	Scanty	Urobilin not increased.
14	0.150	1.00 per cent.	1028	"	3.7 N NaHO	—	—	—	—	Copious	Nil	—	"	"	—
15	0.093	1.00 per cent.	1028	Yellow	3.6 N NaHO	—	One-sixth	30	Fairly increased	—	"	"	"	"	Normal urine + uric acid added (vide No. 12 of Table B.)
16	0.110	1.00 per cent.	1019	"	NaHO	—	One-sixth	10	Slight increase	—	"	"	"	"	—
17	0.060	1.00 per cent.	1020	Deep orange	Acid	3.35	One- fiftieth	20	Not increased	Moderate	Trace	"	"	Copious	—
18	0.064	slight deposit	1028	Yellow	"	2.28	One-tw'nty fifth	12	"	Trace	Nil	"	2.75 per cent.	Scanty	Crystals of uric acid present. Not treated with NaHO before adding HCl.
18a	0.064	0.75 per cent.	1028	Yellow	"	2.28	One-tw'nty fifth	12	"	—	"	"	2.75 per cent.	—	Crystals of uric acid present. Treated with NaHO before adding HCl.
19	0.100	2.00 per cent.	1030	Milk- like	Acid	2.95	One- fifth	30	Increased	Moderate	1.5 per cent.	Nil	"	Copious	Chyle present.
20	0.072	2.00 per cent.	1026	Deep yellow	"	—	—	—	Not increased	—	Nil	—	"	"	Urobilin not increased.
21	0.083	2.00 per cent.	1036	Deep red	"	—	—	—	Slightly increased	Moderately increased	"	"	"	"	Urobilin increased. Free.
22	0.120	2.00 per cent.	1022	Orange	2.5 N NaHO	—	—	10	—	Trace	Trace	"	"	"	—
23	0.080	2.00 per cent.	1027	Deep yellow	2.5 N NaHO	3.49	One-eighth	20	Not increased	Moderate	Nil	"	"	Scanty	—
24	0.080	2.00 per cent.	1023	"	1.2 N NaHO	1.33	One-tw'nty fifth	22	"	Trace	"	"	"	Scanty	—
25	0.100	2.00 per cent.	1038	"	5.0 N NaHO	3.38	One- seventh	30	Moderately increased	Copious	"	"	"	Copious	Copious deposit on cooling
26	0.100	2.00 per cent.	1035	"	5.5 N NaHO	—	—	70	Increased	Trace	"	"	"	Scanty	Urine of same person on different days.
27	0.110	2.00 per cent.	1030	"	Acid	—	—	—	—	—	"	"	"	Copious	Slight deposit on cooling
27a	0.110	2.00 per cent.	1030	"	"	—	—	—	—	—	"	"	"	—	Not treated with NaHO before adding HCl, etc.
28	0.100	Copious deposit	1029	"	"	3.40	One-tenth	40	Moderately increased	Trace	"	"	"	Scanty	NaHO added before HCl and freezing.
29	0.080	"	—	"	—	—	—	—	—	—	"	"	"	"	Not centrifugalized, but left overnight.
30	0.070	"	1034	Yellow	5.0 N NaHO Acid	4.30	One-sixth	24	Not increased	Trace	Nil	"	"	Copious	"
31	0.083	"	1024	"	Acid	2.68	—	26	Copious	—	0.5 per cent.	Trace	"	Scanty	"

deposit is much more dense and reddish-tinged, while that of phosphates and oxalates is cloudy in appearance. However, this deposit of urates does not take place in all cases, even though the amount be increased. Thus, in cases of feebly-acid urine, and when crystals of uric acid are present, no such deposit is formed, although the uric acid may be in excess.

Thus in Table C, 15 out of 31 cases did not give a deposit on simple cooling, although the uric acid was in excess. The acidity of some of these samples was determined by a decinormal solution of caustic soda, with phenolphthalein as an indicator, and in most of the cases where no deposit was obtained by simply lowering the temperature, it was found that about 1 to 2 c.cm. of the caustic-soda solution was required to neutralize 5 c.cm. of the sample of urine.

But this is not the only factor which determines the deposit of urates. Thus in samples Nos. 25 and 26 of Table C of one and the same case, although urates, phosphates, and oxalates were in excess, and the acidity was even high, only a scanty deposit was obtained in one sample, while the other gave a copious deposit. In fact the sample with slightly higher acidity failed to give the deposit. Again, the phosphates were more in the sample with scanty deposit as compared with the other.

The phosphates were estimated by the magnesia mixture method, and the deposit read off after twelve hours. The temperature at which the deposit was looked for was 7° C.

The only other difference between the two samples was an excess of indican in one, which gave a copious deposit of urates. At least, the high acidity failed to produce a copious deposit in one of the samples.

The method of freezing after adding HCl gave a deposit in both cases of about 2 per cent.; thus it is essential to add HCl before freezing the urine.

Again, as has been stated further on, in cases No. 11, 12, 12a, 18, and 18a of Table C, the deposit was of uric acid, and the amount appeared scanty, although the acidity in such cases was high.

Tests with Artificial Urine.

In order to test the accuracy of the method, artificial urine was prepared containing the following constituents: Urea, uric acid, acid phosphate of soda, sodium chloride, and sulphate of soda, dissolved in distilled water by the aid of gentle heat and decinormal solution of sodium hydrate.

The various constituents were in the normal proportions that are found in urine; 5 c.cm. of this were treated with HCl and frozen. Simple addition of HCl brought down some of the uric acid without freezing the mixture, while freezing the mixture after adding the HCl gave a deposit of 0.5 per cent. With a similar artificial urine containing 0.1 per cent. uric acid instead of 0.04 per cent., the deposit obtained with simple HCl was slightly less than 1 per cent., while with HCl and subsequent freezing the amount was 1 per cent. In another artificial urine excess of sulphate of soda was added, but that had no effect on the volume of uric acid deposited. All these samples were feebly acid; about 10 c.cm. of decinormal soda solution were required to neutralize 100 c.cm. of these.

As these artificial samples did not contain any urinary

pigments, and it being likely that these pigments have the property of keeping the urates in solution, I tried the following experiment: I added some measured amount of uric acid to a sample of healthy urine which I knew did not contain an excess of this constituent. The urine was divided into two equal halves; to one half nothing was added, and it was tested by the HCl and freezing method and also by Fokker's method for the amount of actual uric acid; to the other half enough uric acid was added to bring up the total amount to about 0.1 per cent., assuming that the quantity present in the urine was 0.04 per cent. The estimation of uric acid in the first half showed that the assumption was correct. The excess of uric acid was dissolved by gently warming the mixture on a water-bath, no alkali was required to dissolve it. Five cubic centimetres of the pure urine and of this mixture were treated with HCl. in the ordinary way, but no crystals were found to come down in the second tube. These were frozen and centrifugalized, and the normal urine gave a deposit of about 0.5 per cent., while the other one gave a deposit of 1 per cent.

The simple addition of HCl did not give any deposit of crystals even after half an hour. So it is highly probable that the pigments of urine keep the acid in solution.

The actual amount found by Fokker's method was 0.05 per cent., while the amount added was 0.06 per cent.; therefore the quantity of uric acid present in the second half was 0.11 per cent. On the other hand, the two samples mentioned above (Nos. 25 and 26 of Table C) seem to show that perhaps the aromatic sulphates may help to precipitate the urates in ordinary urine. Again, I have not come across a single case where no deposit was obtained on cooling the urine, when both the uric acid and indican were copious, as will be seen by a reference to Table C.

Tests with Animal Urine.

As a further test of the accuracy of this method, dog's urine was used as a solvent for uric acid, and this solution was used as follows:

A. Simple dog's urine was cooled to 0° C., and no deposit was obtained.

B. Same urine was treated with the HCl and freezing method; result was same as in A.

C. Same urine was treated with 0.05 per cent. uric acid and cooled to 0° C., without obtaining any deposit.

D. Urine C was treated with the HCl and freezing method; deposit obtained was 0.5 per cent.

E. Urine A was treated with 0.1 per cent. uric acid and cooled to 0° C.; no deposit.

F. Urine E was treated with HCl and freezing method; deposit was 1 per cent.

As dog's urine is free from uric acid, the deposit obtained in D and F was due to the amount added. The sample used was rich in carbonates and that accounts probably for the absence of deposit in E. The urine was faintly acid and the uric acid was easily dissolved by momentarily boiling the urine.

REMARKS.

From a review of all the observations given in the tables, it seems that in urine with less than 0.05 per cent. uric acid, the deposit is less than 0.5 per cent., while from 0.06 per cent. to 0.15 per cent. the deposit varies between 1 to 2 per cent. This

is rather a wide range, but it is explained by the fact that the soluble urates present in the urine are soon changed into uric acid and biurate of soda. Both these are crystalline in structure, and the deposit of these is a good deal more compact than that of the quadriurates which form a thick, grumous deposit. As the quadriurates commence to change into the other two constituents with varying rapidity, the deposit obtained by the freezing method must necessarily vary within the limits mentioned above.

However, by rendering the urine faintly alkaline and boiling it, etc., as has been described above in the cases of urines which deposit crystals of uric acid, I have been able to reduce the range considerably. Thus for less than 0.04 per cent. uric acid, the deposit is less than 0.5 per cent.; for 0.05 to 0.08 per cent. uric acid, the deposit varies between 0.75 to 1 per cent., and for higher amounts it is 1.5 to 2 per cent.

It will be noticed that the amount of HCl added is very small (about 0.8 per cent.) compared with the amount added in the ordinary clinical methods, in which this acid is used. In fact, I have found that the amount if increased, interferes with the freezing of the urine and the precipitation of the urates.

SUMMARY.

1. Have the urine as free as possible from extraneous dirt.
2. Remove the albumin and pus, if present, by adding acetic acid and heating the urine.
3. Render the urine slightly alkaline by adding tiny drops of strong NaHO—boil it, and take 5 c.cm. of this boiled urine for examination.
4. Add two tiny drops of HCl from a capillary pipette (each drop equals 0.02 c.cm.), stir it well; see that it is quite clear again.
5. Freeze it and centrifugalize till the whole has remelted, and read off the percentage of deposit as soon as the temperature is about 25° C.

In conclusion I have to thank the Government of Bombay for having given me a grant for this research.

